Egypt, J. Plast. Reconstr. Surg., Vol. 32, No. 2, July: 175-182, 2008

Effect of Depth of Early Burn Wound Excision on the Alteration of Immunological Profile in Severe Burns

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ABSTRACT

Surgical excision of the burn wound in resuscitated patients especially when done early, results in improvement in survival rates and morbidity. The study of the alteration of the immunological profile in relation to timing and extent of early excision has been established. However, the impact of the depth of escharectomy on the immunological profile changes has not yet been studied. This study compared the impact of the two different techniques Escharectomy on alteration of the immunological profile in severe burns. The study was conducted in the Burn Unit of Ain Shams University Hospitals, on 30 acutely burned adult patients, who had deep dermal burns with surface area ranged between 21 and 70%. They were divided into two groups: First group: 15 patients who were candidates for tangential excision and Second group: 15 patients who were candidates for down-to fascia excision. Using IL-6 and TNF-α serum assay as indicators for immunological profile of the patients, analysis of data revealed insignificant statistical variation in levels of IL-6 assay of neither survivors nor non-survivors of both groups at the different time points (p-value >0.4376, p-value >0.4198 respectively). The same results were concluded for TNF-α assay of survivors and non-survivors of both groups (p-value >0.4376, p-value >0.5806 respectively). It is concluded that in deep dermal burns, there is no advantage to routinely performing a fascial excision, since the immunological profile changes are statistically comparable to those in tangential excision.

INTRODUCTION

The burn wound is the source of virtually all ill effects, local and systemic, seen in a burned patient. Burn eschar exerts a systemic immune response that cascades through cytokine pathways leading to Systemic Inflammatory Response Syndrome (SIRS), which may progress to Multiple Organ Failure (MOF) **[1,2]**. In addition, eschar acts as a nidus for infection that is aggravated by immune suppression state. This may progress to sepsis or sepsis-induced SIRS **[2]**.

Level of tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) may be considered to be the most important poor prognostic factors related to SIRS and MOF following thermal injury **[3,4]**.

The ideal depth of burn wound excision has not yet been established **[3]**. However, theoretically, injured tissue following thermal trauma presents a central area of necrosis surrounded by a stasis zone in which cell metabolism is slowed down, creating favorable conditions for wound sepsis **[5]**. The bacterial colonization is responsible for the deepening of the wounds by lysis of the surrounding healthy tissue. Episodes of sepsis lead to ischemic necrosis of subcutaneous fat subsequent to poor peripheral perfusion and microvascular stasis, that leads to late graft loss and these ischemic areas become portals for invasive wound sepsis **[5,6]**.

It is hypothesized that the accumulation and reabsorption of subeschar tissue fluid (STF) may increase the morbidity and mortality rates in severely burned patients. Therefore, the unburned tissues at the margin and the depth of the burn may be affected and may exaggerate the systemic inflammation **[7]**.

The study of the alteration of the immunological profile in relation to timing and extent of early excision has been established **[5,6,9,12]**. However, the impact of the technique of early burn wound excision on the immunological profile changes has not yet been studied.

The aim of this study is to compare the effect of two different techniques of early burn wound excision (tangential excision and down-to-fascia excision) on alteration of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels as indicators for the immunological profile alterations.

PATIENTS AND METHODS

This prospective comparative study was conducted in the Burn Unit of Ain Shams University Hospitals in the period from January 2004 until

March 2006. The study included 30 acutely burned adult patients. All patients had a combination of superficial and deep dermal burns. The burned surface area (BSA) ranged between 21 and 70% according to the Lund and Browder chart. The deep areas were ranging between 15 and 25% of TBSA.

Patients were admitted within 8 hours from the injury. Patients with preexisting medical diseases (e.g. renal or liver impairment, diabetes mellitus, immunodeficiency syndrome as AIDS, leukemia, lymphoma, lymphocytopenia) were excluded.

The 30 patients were divided into two groups according to the technique of the excision:

First group: Included 15 patients who were candidates for tangential early burn wound excision and application of allograft within the first five postburn days (PBDs).

Second group: Included 15 patients who were candidates for down-to fascia early burn wound excision and application of allograft within the first five PBDs.

Management protocol: All patients were weighed in the admission room, prior to resuscitation and the following protocols were applied:

I- *Resuscitation protocol:*

Resuscitation was performed using the Modified Parkland Formula. The success of resuscitation was assessed by monitoring pulse rate, blood pressure, urine output per hour, and central venous pressure. Throughout resuscitation, alterations could be made on the amount of fluids given, depending upon the hemodynamic status of the burned patient. In the first 24 hours, the only solution given was Ringer's Lactate. In the following days, a combination of crystalloids and colloids was given and blood transfusion, fresh frozen plasma, and albumin, were given according to the laboratory data.

II- *Nutritional protocol:*

Caloric and protein requirements were calculated according to the Curreri formula: Total caloric requirements/day = 25 x weight in kg + 40 x % BSA. Feeding was started within 24 hours from the time of admission.

III- *Post-resuscitation management:*

Wound management:

Burn wounds were cleansed on admission using aqueous povidone iodine (10%). Superficial and deep dermal burns were dressed with tulle grass. The change of dressing was performed on a daily or twice daily basis.

Surgical procedures (early excision and grafting):

- Timing of excision: Early excision and grafting was attempted once the resuscitation has been accomplished, and the patient's general condition has become stable. The burn wound excision was started within first five PBDs and it was completed by the eleventh PBD.
- Extent of excision: Extent of excised area ranged between 5% and 16% of TBSA per session.
- Techniques and depth of excision:
  - a- *Tangential excision:* The tangential method-sequentially shaving the eschar from the wound surface until a viable-tissue plane-was applied for the first patient group. An acceptable wound bed was identified by active punctuate bleeding. The procedure was usually performed using a hand-held blade equipped with a calibrated depth guard (0.010-0.025 inches).
  - b- *Down to fascia excision:* Linear escharotomies were placed 180º apart on a limb, and/or at the wound margins otherwise, which was limited at the level of wrist or ankle. The procedure was usually performed using a scalpel or electrocautery unit.

The total areas excised were covered by allograft (homograft or amniotic membranes) except case no. 7 in first group and cases no. 1,9 and 15 in second group, where autografts were used.

Monitoring of the patients:

Organ dysfunction was based on the following criteria:

Clinical monitoring:

I- *Systemic monitoring:* Alteration in the level of consciousness, changes in temperature; severe arrhythmias; anuria, oliguria and tachypnea, orthopnea and/or cyanosis, assisted ventilation for more than 5 days.

II- *Local (burn wound) monitoring:* Local signs suggestive of burn wound infection; progression of partial-thickness to full-thickness injury; change in wound color (focal areas of red, brown, or black discoloration) or green discoloration of the subcutaneous fat.

Laboratory monitoring:

All laboratory tests were assessed at one day before operation and 3rd, 7th and 14th days after escharectomy.

Routine laboratory investigations: Complete blood count (CBC), Coagulation profile, Random blood sugar, Serum albumin (Alb), C-reactive protein.

Specific Lab investigations were done for detection of organ dysfunction [20]; arterial blood gases (PaO_2 <50mmHg or SaO_2 <90%); Serum creatinine (>1mg/d) and/or Blood urea nitrogen (BUN >15mg/100mL); SGOT (male: >46u/L, female: >35u/L), SGPT (male: >46u/L, female: >35u/L) and creatine phosphokinase (CPK >200U/L).

Assay of serum levels of cytokines: This was done in Ain Shams University Hospital Laboratories (Immunity Lab):

Interleukin-6 (IL-6) assay: Was done by an immunoenzymometric assay for the quantitative measurement of human IL-6 in serum (EASIA) (Biosource Europe S.A., Belgium). The detection limits were 80~2024pg/mL for IL-6 assay.

Tumor necrosis factor-alpha (TNF-α) assay: Was done by an immunoenzymometric assay for the quantitative measurement of human TNF-α in serum (EASIA) (Biosource Europe S.A., Belgium). The detection limits were 50~1800pg/mL for TNF-α assay.

To compare the effect of the technique of burn wound excision on the immunological profile changes, the following comparisons were done:

- Serum IL-6 assay levels in survivors of both groups.
- Serum IL-6 assay levels in non-survivors of both groups.
- Serum TNF-α assay levels in survivors of both groups.
- Serum TNF-α assay levels in non-survivors of both groups.

Mean ± Standard Deviation (Mean ± SD) and probability indices (*p-values*) according to Mann-Whitney test (independent samples), a *p-value* less than 0.05 was considered statistically significant.

RESULTS

The burned surface area (BSA) ranged between 21 and 70% according to the Lund and Browder chart (42.8±13.4%). The deep areas, (i.e. deep dermal) were ranging between 15 and 25% of TBSA, (21.9±2.9%). Out of the 30 patients, 25 had flame burns (83%), 4 had scalds (13%) and 1 had flash burns (3%). The shortest hospital stay was 8 days, and the longest stay was 39 days, (20.9±10 days).

Patient population of first group:

This group included 6 males and 9 females. Their ages ranged between 50-20 years, (29.9±11 years). The total burned surface area (BSA) ranged between 23-60% of total body surface area (TBSA), (42±9%).

The deep areas, (i.e. deep dermal) were ranging between 15 and 25% of TBSA, (22±2%). Out of these 15 cases, 13 had flame burns (87%) and 2 had scalds (13%). The mortalities were 6 of 15 (40%), 1 case with single organ failure and 5 cases with MOF.

Patient population of second group:

This group included 9 males and 6 females. Their ages ranged between 45-20 years, (28.5±7.3 years). The total burned surface area (BSA) ranged between 21-70% of total body surface area (TBSA), (44±16%).

The deep dermal areas were ranging between 15 and 25% of TBSA area, (21.7±3.5%). Out of these 15 cases, 12 had flame burns (80%) 2 had scalds (13%) and 1 had flash burns (7%). The mortalities were 10 of 15 (66.7%), 3 case with single organ failure and 7 cases with MOF.

Analysis of laboratory investigations:

All patients had high levels of all blood elements in the preoperative samples (with first five PBDs) (due to haemoconcentration) that began to resolve after proper fluid therapy. Total leucocytic count was elevated in all patients without clinical manifestations of infection. Thrombocytosis occurred in 4 patients in first group and 7 patients in second group who had MOD. Elevated liver transaminases were noticed in 5 patients (4 patients had MOD and 1 patient had single organ dysfunction) in first group and 8 patients in second group. C-reactive protein and creatine phosphokinase (CPK) values were high in 4 patients in first group and 7 patients in second group. Albumin, fasting blood sugar, serum creatinine and blood urea/nitrogen (BUN) levels were within the normal values throughout the study.

Assessment of serum levels of cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were done (Tables 1,2).

Table (1): Cytokines assay (pg/ml) results of first group at different time points.

Patients	Preoperative day		3rd Postoperative day		7th Postoperative day		4th Postoperative day	
	IL-6	TNF-α	IL-6	TNF-α	IL-6	TNF-α	IL-6	TNF-α
1**	700	490	650	400	2024	1400	–	–
2*	200	300	500	450	2024	700	1000	200
3**	570	340	200	300	850	1600	–	–
4*	500	350	2024	500	700	500	450	100
5*	600	480	1000	550	1500	700	1000	200
6**	500	350	450	400	1300	1200	2024	1800
7*	600	360	650	500	200	300	150	150
8*	400	240	2024	300	430	240	400	160
9*	600	480	1000	400	700	320	450	220
10*	800	640	600	500	510	300	200	130
11*	700	420	510	400	1000	400	300	100
12*	600	360	450	450	1000	200	100	80
13**	1000	700	2024	900	1700	1400	2024	1200
14**	1250	1000	650	1200	2024	1800	–	–
15**	620	500	700	700	2024	1800	–	–

* Survivor. ** Non-survivor.

Table (2): Cytokines assay (pg/ml) results of second group at different time points.

Patients	Preoperative day		3rd Postoperative day		7th Postoperative day		4th Postoperative day	
	IL-6	TNF-α	IL-6	TNF-α	IL-6	TNF-α	IL-6	TNF-α
1*	300	180	510	460	1000	360	510	250
2**	600	360	600	400	350	900	1450	1400
3**	1200	840	2024	400	1900	1200	2024	1400
4**	1000	600	1100	500	700	800	2024	1200
5**	500	400	510	520	800	900	510	1400
6**	800	560	350	720	800	1200	–	–
7*	500	300	200	500	250	1600	300	1600
8**	300	180	500	500	2024	1800	2024	1800
9**	700	420	600	504	900	1600	–	–
10**	1250	1000	2024	600	2024	900	–	–
11*	570	400	510	400	1000	640	800	100
12**	620	370	1300	500	2024	1600	–	–
13*	600	360	2024	600	400	260	200	200
14*	570	340	700	350	400	380	100	200
15*	650	520	350	300	600	370	200	100

* Survivor. ** Non-survivor.

Analysis of laboratory results of first group:

• *Comparison between serum IL-6 assay levels of survivors and non-survivors of first group:*

Analysis of data revealed significant higher levels of IL-6 assay of non-survivors compared to survivors at 7th (896±568pg/ml for survivors, 1654± 486pg/ml for non survivors) and 14th (450± 336pg/ml for survivors, 2024±0pg/ml for non survivors) postoperative days (*p*-value=0.0360, 0.0004 respectively).

• *Comparison between serum TNF-α assay levels of survivors and non-survivors of first group:*

Analysis of data revealed significant higher levels of TNF-α assay of non-survivors compared to survivors at 7th (406±187pg/ml for survivors, 1533±242pg/ml for non survivors) and 14th (148± 50pg/ml for survivors, 1500±424pg/ml for non survivors) postoperative days (*p*-value= 0.0004, 0.0004 respectively).

Analysis of laboratory results of second group:

• *Comparison between serum IL-6 assay levels of survivors and non-survivors of second group:*

Analysis of data revealed significant higher levels of IL-6 assay of non-survivors compared to survivors at 7th (680±303pg/ml for survivors, 1177± 730pg/ml for non survivors) and 14th (362± 289pg/ml for survivors, 1643±796pg/ml for non survivors) postoperative days (*p*-value = 0.0710, 0.0027 respectively).

• *Comparison between serum TNF-α assay levels of survivors and non-survivors of second group:*

Analysis of data revealed significant higher levels of TNF-α assay of non-survivors compared to survivors at 7th (402±141pg/ml for survivors, 1250±371pg/ml for non survivors) and 14th (170± 67pg/ml for survivors, 1466±206pg/ml for non survivors) postoperative days (*p*-value <0.0001, <0.0001 respectively).

Comparison between IL-6 assay levels of survivors in both groups (Chart 1):

Analysis of data revealed no significant variations in levels of IL-6 assay of first group survivors compared to second group survivors (*p*-value >0.4376).

Comparison between TNF-α assay levels of survivors in both groups (Chart 2):

Analysis of data revealed no significant variations in levels of TNF-α assay of first group survivors compared to second group survivors (*p*-value >0.4376).

Comparison between IL-6 assay levels of non-survivors in both groups (Chart 3):

Analysis of data revealed no significant variations in levels of IL-6 assay of first group non-survivors compared to second group survivors (*p*-value >0.4198).

Comparison between TNF-α assay levels of non-survivors in both groups (Chart 4):

Analysis of data revealed no significant variations in levels of TNF-α assay of first group non-survivors compared to second group survivors (*p*-value >0.5806).

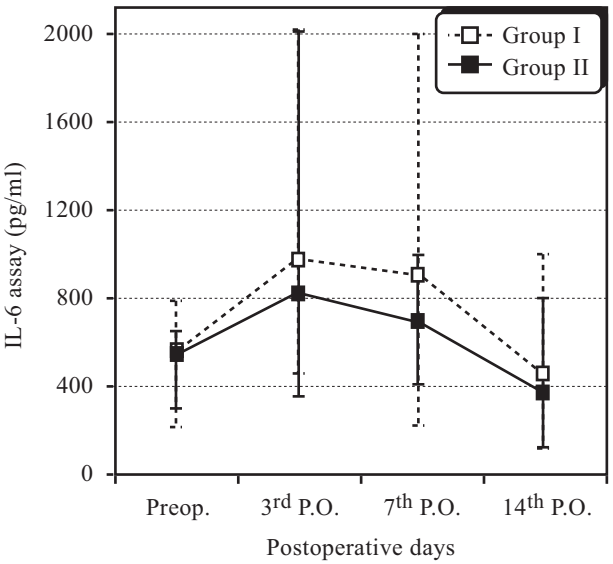

Chart (1): IL-6 assay levels of survivors in both groups at preoperative, 3rd, 7th and 14th postoperative (PO) days.

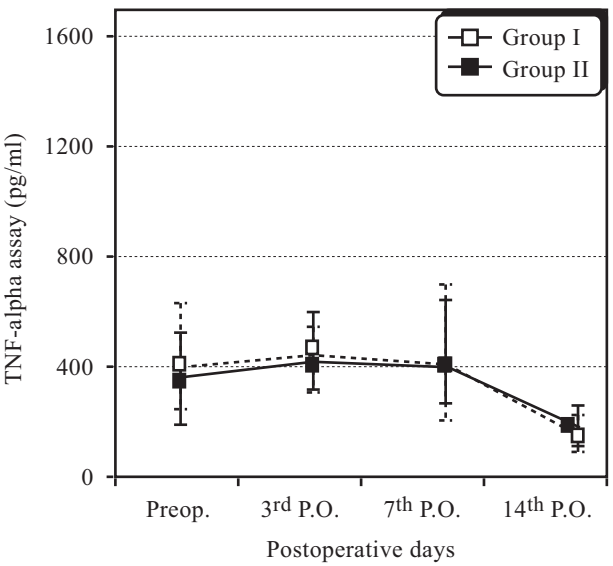

Chart (2): TNF-α assay levels of survivors in both groups at preoperative, 3rd, 7th and 14th PO days.

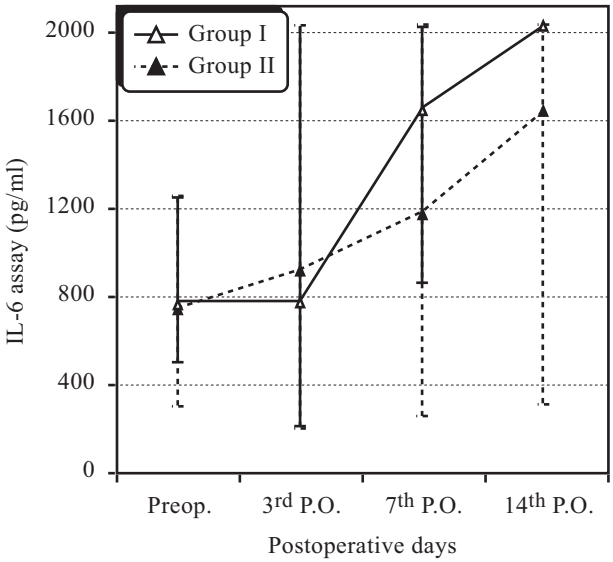

Chart (3): IL-6 assay levels of non-survivors in both groups at preoperative, 3rd, 7th and 14th PO days.

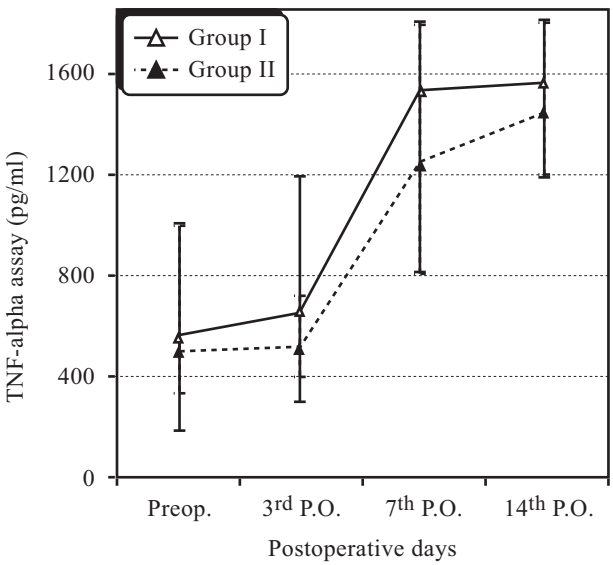

Chart (4): TNF-α assay levels of non-survivors in both groups at preoperative, 3rd, 7th and 14th PO days.

DISCUSSION

It is generally accepted nowadays that the primary effect of thermal injury, is to trigger the immune system, thus resulting in a local and a systemic inflammatory response. This effect occurs primarily through a heat induced eschar-derived toxin lipoprotein complex (LPC). The LPC-mediated systemic inflammation might eventually bring about dysfunction then failure of vital organs. Thus, it is clear that, in severe burns, mortality can occur as a consequence of the LPC-mediated systemic inflammation, even before the occurrence of infection **[10]**. Alternatively, the burn toxin might result in a paradoxical immune-suppression, thus allowing burn wound infection to come to the scene. The effect of infection is eventually to trigger the immune system, thus resulting in a SIRS through the liberation of bacterial toxins, whether the endotoxin (lipopolysaccharide, LPS) of gram-ve rods, or the exotoxin of gram+ve species. Consequently, the early excision of burn eschar restores cellular and humoral immunity and modulates the stress response in burned patients that leads to reduce the incidence of SIRS and MOF **[4]**.

The depth of early burn wound excision has been always controversial. Two techniques were adopted for the escharectomy in burned patients. These techniques include tangential and fascial excisions. The tangential excision technique has the advantages of preserving subdermal fat over bony prominences for cosmetic reasons, as well as maintaining the integrity of lymphatic drainage and cutaneous nerves. However, the grafting onto subdermal fat results in a lower success. Exaggerated blood loss is another disadvantage of this technique. Conversely, Down-to-fascia excision assures a viable bed for skin grafting with moderate blood loss, especially if done under tourniquet control. The major disadvantages include damage to lymphatics and cutaneous nerves, loss of subcutaneous fat, which compromise the cosmetic outcome **[11]**.

Chen **[7]** suggested that the accumulation and reabsorption of subeschar tissue fluid (STF) likely contributes to the serologic evidence of cell mediated immunological abnormalities documented in severe thermal injury and may increase the morbidity and mortality rates in severe thermal injuries. Therefore, we aimed at finding a possible impact of depth of burn wound excision on the immunological profile and hence the clinical outcome in severely burned patients.

Concerning laboratory results, serum levels of IL-6 and TNF-α were used to compare between survivors and non-survivors in each group, between survivors in both groups and between no-survivors in both groups. As regards, IL-6 assay for comparison between survivors and non-survivors of the same group, the results showed significantly higher levels of IL-6 assay of non-survivors compared to survivors in both group at 7th and 14th postoperative days (for first group, *p*-values=0.0360 and 0.0004 respectively, and for second group, *p*-values=0.0710 and 0.0027 respectively).

There has been always a controversy in the correlation between the IL-6 serum levels and

mortality rate, in severely burned patients. Rodriguez **[12]** reported no association between mortality and IL-6. Conversely Munster **[3]** suggested that low serum level of IL-6 might be considered one of the most important poor prognostic factors related to SIRS following thermal injury. A similar conclusion was reached out in the study of Deveci **[13]**, who stated that IL-6 inhibits the severity of the inflammatory response in the early period of thermal injury by decreasing serum levels of TNF-α. However, Hack **[14]** and Drost **[15]** found a correlation between high IL-6 serum levels and incidence of MOF and consequently mortality rates in severe thermal injuries.

In this study, TNF-α assay for comparison between survivors and non-survivors of the same group was verified. Our results conformed with the findings of previous authors who emphasized that there were significant higher levels of TNF-α assay of non-survivors compared to survivors in both group at 7th and 14th postoperative days, (for first group *p*-values=0.0004, 0.0004 respectively, and for second group *p*-values <0.0001, <0.0001 respectively). Wherever, Munster **[3]** suggested that high serum level of TNF-α might be considered one of most important poor prognostic factors related to MOF following thermal injury. Conversely, Rodriguez **[12]** reported no association between mortality and tumor necrosis factor-alpha (TNF-α).

By comparing the curves of mean values of IL-6 assay and TNF-α assay in survivors in both groups, we found that burn wound excision normalized TNF-α serum levels, in both groups. The elevated serum levels of IL-6 were also decreased by burn wound excision, but they did not reach the normal levels. These results were comparable to the findings of Schwacha et al. **[16]**, who found that burn wound excision and grafting normalized TNF-α production.

Concerning impact of depth of excision on serum levels of IL-6 and TNF-α, analysis of data revealed insignificant variation in levels of IL-6 assay in both groups neither in survivors nor non-survivors at different time points (*p*-value >0.4376, *p*-value >0.4198 respectively). Similarly, there was insignificant variation in levels of TNF-α assay between both groups neither in survivors nor non-survivors at different time points (*p*-value >0.4376, *p*-value >0.5806 respectively).

Consequently, these findings disagreed with the concept of "fascial excision may be better than tangential excision because it removes large amounts of subeschar tissue fluid". Conversely, many studies aimed at the evaluation of the role of subeschar tissue fluids (STF) in development of MOF. Wherever, Ferrara et al. **[17]** and Dyess et al. **[18]** suggested that: Subeschar tissue fluid may act as both an immunologic barrier to microbial clearance in otherwise viable subcutaneous tissue and a reservoir for systemically reabsorbed immuno-suppressive factors. In addition to removing dead tissue, fascial excision may prove beneficial because it removes large amounts of immuno-suppressive subeschar tissue fluid. Similar findings were encountered in the study of Chen et al. **[7]** who suggested that subeschar tissue fluid might be one of the inducing factors involved in the genesis of SIRS and the development of MOF in the early postburn stage. In addition, it was concluded that subeschar tissue fluid might induce the development of SIRS in rats, which further lead to the development of MOF. However, Rong et al. **[19]** stated that the cells and large molecules seems to be more difficult to enter subeschar tissue fluid compared with small molecules and no marked local inflammatory response occurs in subeschar tissue fluid during early stage of severe burn, and subeschar tissue fluid has no lethal effect.

It is concluded that in deep dermal burns, following initial evaluation, wound excision is carried beyond the deepest level of injured tissue, where fascial excision is used for full-thickness injury and tangential excision is used in or below the dermis for deep dermal injury.

Acknowledgment:

We would like to express our immense debts to Prof. Dr. Ikram Safe Head of the Plastic and Reconstructive Surgery Department, Faculty of Medicine, Ain Shams University, for his concrete and psychological support.

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